PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : A61L 9/04, A61K 9/12	A1	(11) International Publication Number: WO 96/25184
		(43) International Publication Date: 22 August 1996 (22.08.96)

(21) International Application Number: PCT/US96/01745

(22) International Filing Date: 8 February 1996 (08.02.96)

(30) Priority Data:
08/389,304   16 February 1995 (16.02.95)   US

(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors: ZAPOL, Warren, M.; 182 Holden Wood Road, Concord, MA 01742 (US). BLOCH, Kenneth, D.; Apartment 313, 20 Webster Street, Brookline, MA 02146 (US).

(74) Agent: FRASER, Janis, K.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: TREATMENT OF VASCULAR THROMBOSIS AND RESTENOSIS WITH INHALED NITRIC OXIDE

(57) Abstract

Disclosed are methods of treating, inhibiting or preventing vascular thrombosis or arterial restenosis in a mammal. The disclosed methods include causing the mammal to inhale a therapeutically-effective concentration of gaseous nitric oxide (NO). Also disclosed are methods that include the administration of the following types of agents in conjunction with inhaled nitric oxide: compounds that potentiate the beneficial effects of inhaled nitric oxide, and antithrombotic agents that complement or supplement the beneficial effects of inhaled nitric oxide. The figure is a graph comparing the intima/media ratio in control rats, rats exposed for two weeks to 20 ppm NO, and rats exposed for one week to 80 ppm NO, followed by one week of breathing air. The asterisk indicates values significantly differing from baseline (p < 0.05).

TREATMENT OF VASCULAR THROMBOSIS AND RESTENOSIS WITH INHALED NITRIC OXIDE

Statement as to Federally Sponsored Research

Background of the Invention

10   This invention relates to methods of treating, inhibiting or preventing vascular thrombosis and methods of treating, inhibiting or preventing arterial restenosis resulting from excessive intimal hyperplasia.

A thrombosis, i.e, the formation or presence of a

15 blood clot within a blood vessel, may result from physical injury of an arterial wall by a vascular interventional procedure such as percutaneous transluminal coronary angioplasty ("PTCA"; a type of balloon angioplasty) or coronary bypass surgery.

20 Thrombosis may also result from progression of a natural disease, such as atherosclerosis. Various agents, including aspirin, prostaglandin E_1, selective thromboxane A_2 inhibitors, selective thrombin inhibitors, platelet receptor GPIIb/IIIa blockers, tissue plasminogen

25 activator, streptokinase, heparin and kistrin have been used as antithrombotics. See, e.g., Yasuda et al. (Circulation 83:1038 (1991)); and Gold et al., (Circulation (Supplement IV) 83:IV26 (1991)). Currently available antiplatelet agents include aspirin,

30 ticlopidin, monoclonal antibodies, nitroglycerin and sodium nitroprusside.

Another unwanted result of arterial injury from vascular interventional procedures is arterial restenosis. For example, PTCA has been used extensively

35. to open occluded arteries in both the coronary and peripheral vascular systems. Although initially

successful in ver 95 percent f cases, a gradual
renarr wing r reocclusion process, known as restenosis,
occurs within six months in 30 to 50 percent of the
patients.

5   Vascular interventional procedures tend to damage
the vascular wall. Such injury triggers the
proliferation of vascular smooth muscle cells, resulting
in intimal hyperplasia. This proliferation may be due in
part to the effects of platelet-derived growth factors
10 released by platelets which adhere to the site of
arterial injury. Excessive intimal hyperplasia leads to
restenosis, i.e., a re-narrowing of the arterial lumen.
Thus, arterial restenosis can severely limit the long-
term effectiveness of vascular interventional procedures.

15   Summary of the Invention
        It has been discovered that inhaled nitric oxide
(NO) gas increases blood vessel patency after lysis of a
thrombus at the site of a critical stenosis, without
causing any systemic hemodynamic effects. The
20 antithrombotic effects of inhaled NO persist after the
cessation of NO inhalation. It has also been discovered
that inhaled NO inhibits arterial restenosis resulting
from excessive intimal hyperplasia.
        Accordingly, in one aspect, the invention features
25 a method for treating, inhibiting or preventing a
thrombosis in a mammal, by causing the mammal to inhale a
therapeutically effective amount of gaseous NO. The
thrombosis may be associated with a disease or injury.
        In another aspect, the invention features a method
30 for treating, inhibiting or preventing arterial
restenosis in a mammal, by causing the mammal to inhale a
therapeutically effective amount of gaseous NO. The
arterial restenosis may be associated with a disease or
injury.

A second c mpound which potentiates the beneficial
effects of inhaled NO may be administered to a mammal, in
conjunction with NO inhalation. One means whereby the
second compound could potentiate the beneficial effects
5 of NO inhalation is by prolongation of the effect of
inhaled NO in target tissues, or cells that interact with
the target tissues, without causing undesirable systemic
vasodilation and concomitant decrease in systemic blood
pressure. More particularly, prolonging the
10 antithrombotic and/or intimal hyperplasia-inhibiting
effect of inhaled NO may be accomplished by administering
a phosphodiesterase-inhibiting agent in conjunction with
(i.e., before, during or immediately after) NO
inhalation. Preferred phosphodiesterase inhibitors are
15 those that selectively inhibit cGMP-specific
phosphodiesterases, while minimally affecting the
breakdown of adenosine 3',5'-cyclic monophosphate (cAMP)
by other phosphodiesterases. A preferred
phosphodiesterase inhibitor displaying the aforementioned
20 specificity is Zaprinast" (M&B 22948; 2-o-propoxyphenyl-
8-azapurine-6-one, Rhone-Poulenc Rorer, Dagenham Essex,
UK). Other cGMP-specific phosphodiesterase inhibitors
are known and may be substituted for Zaprinast".
        Another means whereby the second compound could
25 potentiate the beneficial effects of NO inhalation is by
prolongation of the in vivo half-life of NO. That may be
accomplished, for example, by administering superoxide
dismutase. Superoxide dismutase removes superoxide
radicals, which react with NO.
30   In other preferred embodiments, a second
antithrombotic agent which augments the beneficial
antithrombotic effects of inhaled NO may be administered
to a mammal in conjunction with the inhaled NO. Examples
include aspirin, ticlopidine, monoclonal antibodies,
35 nitroglycerin, and sodium nitroprusside.

As used herein, "acute isch mic cor nary syndrome" means acute cclusion of a coronary artery, e.g., acute myocardial infarction, unstable angina, crescendo angina, angina pectoris, coronary intermediate syndrome, angina

5   pectoris status post thrombolysis and angina pectoris status post PTCA.

As used herein, "antithrombotic agent" means an agent that inhibits thrombus formation, stimulates thrombolysis, i.e., thrombus dissolution, or both.

10   As used herein, "intimal (or neointimal) hyperplasia" means proliferation of arterial smooth muscle cells in the intima, in response to arterial endothelial denudation.

As used herein, "revascularization" means

15   reestablishment of blood supply to a part of the mammalian body.

As used herein, "target cells" means non-pulmonary cells that interact directly with NO, following NO inhalation, to mediate a beneficial effect in the

20. inhibition, prevention or treatment of thrombosis or arterial restenosis.

As used herein, a "therapeutically effective concentration of gaseous NO" is a concentration that induces one or more of the following in a given patient:

25   relief of chest pain, relief of shortness of breath, resolution of ischemic electrocardiographic changes, and improved coronary artery blood flow (which may be shown by angiography).

As used herein, a "therapeutically effective"

30   amount of a phosphodiesterase inhibitor is an amount that increases the duration (i.e., half-time) or magnitude of the therapeutic effect of gaseous NO.

As used herein, "thrombosis" means the formation or presence of a blood clot within a blood vessel, which

clot may caus infarction or ischemia of tissu s supplied
by the v ssel.

As used herein, "arterial restenosis" means an
abnormal narrowing of an arterial lumen as a result of
5 excessive (neo)intimal hyperplasia.

As used herein, "vascular interventional
procedure" means any surgical procedure that involves an
anatomical disruption or a mechanical disturbance of a
blood vessel. A non-exclusive list of vascular
10 interventional procedures includes balloon angioplasty,
laser angioplasty, coronary artery surgery, atherectomy
and coronary artery stents.

The methods herein disclosed are useful for
treating, inhibiting or preventing thrombosis, such as
15 may result from a naturally occurring disease, e.g.,
athrosclerosis, or from a vascular interventional
procedure, e.g., angioplasty or coronary bypass surgery.
The methods herein disclosed are also useful for
treating, inhibiting or preventing arterial restenosis
20 resulting from excessive intimal hyperplasia. Such
arterial restenosis is a frequent complication associated
with vascular interventional procedures, such as PTCA or
coronary bypass surgery.

According to this invention, NO gas may be
25 administered:

(a) continuously or intermittently;

(b) in the absence of tobacco smoke;

(c) at a concentration between 0.1 and 300 parts
per million (ppm), preferably between 1.0 and 200 ppm,
30 and most preferably between 20 and 100 ppm;

(d) as a mixture including NO, oxygen (O_2), and
nitrogen (N_2) or other inert gases, preferably having an
FiO_2 (i.e., proportion of O_2, by volume) of 0.20 to 0.99,
the proportion of O_2 in air being 0.21, and where the NO
35 gas is mixed with the O_2-containing gas shortly before

inhalation (e.g., less than 5 minutes before inhalation,
and preferably less than one minute before inhalation);
and

       (e) in such a way that the concentration of NO_2 is
5 monitored and kept within safe limits (e.g., less than 1
ppm).

       An important advantage of this invention is that
inhaled NO does not induce systemic vasodilation and
concomitant acute systemic hypotension. Systemic
10 vasodilation is an undesirable, potentially dangerous
side effect associated with sustained systemic NO release
from oral or intravenously administered NO donor
compounds which have been used to inhibit thrombosis or
excessive intimal hyperplasia.

15   Other features and advantages of the invention
will be apparent from the following description of the
preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

      Fig. 1A is a graph comparing open arterial time
20 (as a percentage of total time) for the control group
before, during and after zero ppm NO inhalation, in the
canine coronary artery thrombosis model.

      Fig. 1B is a graph comparing open arterial time
(as a percentage of total time) for the 20 ppm inhaled NO
25 group before, during and after NO inhalation, in the
canine coronary artery thrombosis model. The asterisk
indicates value significantly differs from baseline ($p <$
0.05).

      Fig. 1C is a graph comparing open arterial time
30 (as a percentage of total time) for the 80 ppm inhaled NO
group before, during and after NO inhalation, in the
canine coronary artery thrombosis model. The asterisk
indicates value significantly differs from baseline ($p <$
0.05).

Fig. 1D is a graph comparing open arterial time (as a percentage of total time) for the 200 ppm inhaled NO group before, during and after NO inhalation, in the canine coronary artery thrombosis model.

5   Fig. 2 is a graph showing ADP-induced canine platelet aggregation curves for samples exposed to 0 ppm NO or 400 ppm NO.

Fig. 3 is a graph summarizing dose response data on the effect of inhaled NO on canine platelet

10   aggregation.

Fig. 4 is a graph comparing the intima/media ratio in control rats; rats exposed for two weeks to 20 ppm NO; rats exposed for two weeks to 80 ppm NO; and rats exposed for one week to 80 ppm NO, followed by one week of

15   breathing air. The asterisk indicates value significantly differs from baseline (p < 0.05).

Detailed Description

Thrombosis

The invention provides a simple, rapid, selective

20   and efficacious method for treating, inhibiting or preventing a vascular thrombosis in a mammal, such as a human patient. The thrombosis to be treated, inhibited or prevented may result from any of the various causes of thromboses. For example, the type of thrombosis may be a

25   posttraumatic arterial thrombosis. The thrombosis may be at any one of various locations in the mammalian body. For example, the thrombosis location may be arterial, venous, coronary, cerebral, femoral, renal or placental. Preferably, the location of the thrombosis treated,

30   inhibited or prevented is not pulmonary.

Coronary thromboses are often associated with an acute ischemic coronary syndrome. Any thrombosis-related acute ischemic coronary syndrome may be treated, inhibited or prevented by the administration of inhaled

- 8 -

NO, acc rding to this inventi n. The thr mbosis-related
acute ischemic c r nary syndr me may be associated with,
e.g., an artery-occluding disease or a vascular
interventional procedure. Thrombosis-related acute
5 ischemic coronary syndromes include, but are not limited
to, myocardial infarction, unstable angina pectoris,
thrombosis after coronary revascularization, and
reocclusion after coronary thrombolysis. Coronary
revascularization may be accomplished by various known
10 vascular interventional procedures, e.g., PTCA, laser
angioplasty, coronary artery bypass grafting, coronary
artery atherectomy or coronary artery stents.

Inhaled NO may be used, according to this
invention, to treat a mammal that has been identified as
15 having an existing thrombosis. Mammals, and humans in
particular, are known to display various signs and
symptoms relating to the existence of a thrombosis, and
may be identified thereby. The recognition of such signs
and symptoms is within the skill of medical
20 practitioners. Signs and symptoms of a thrombosis in a
human patient include, but are not limited to, the
following: chest pain, shortness of breath, paralysis,
limb pain, myocardial infarction, angina pectoris,
unstable angina pectoris, crescendo angina pectoris,
25 ischemic congestive heart failure, cardiogenic shock,
peripheral vascular disease and ischemic limb. In one
embodiment of this invention, the identified mammal is a
human who has a thrombosis, and the thrombosis to be
treated by NO inhalation is manifested as a myocardial
30 infarction.

Alternatively, inhaled NO also may be used to
inhibit or prevent thrombosis development in a mammal
that does not have an existing thrombosis but has been
identified as being at risk of developing a thrombosis.
35 Certain existing pathological conditions, e.g.,

ather scl r sis, are kn wn to put an individual at risk
of devel ping a thrombosis. In additi n, certain
vascular interventional procedures, e.g., balloon
angioplasty and coronary artery bypass surgery, are known
5 to put an individual at risk of developing a thrombosis.
Accordingly, individuals diagnosed as having pathological
conditions with which an increased risk of thrombosis is
associated, individuals who have undergone one or more
vascular interventional procedures, and individuals for
10 whom one or more vascular interventional procedures are
imminent, may be advantageously treated according to this
invention.

        Similarly, the identified mammal may be a human at
risk of developing a thrombosis, where the risk is
15 associated with an artery-occluding disease such as
atherosclerosis. Individuals with such pathological
conditions may be identified by methods known to medical
practitioners trained in the relevant areas of medical
practice.

20   In another embodiment of this invention, the risk
is associated with a vascular interventional procedure
such as angioplasty. In a particularly preferred
embodiment, the identified mammal is a human at risk of
developing a thrombosis as a result of a PTCA procedure.

25 Arterial Restenosis

        The invention provides a simple, rapid, selective
and efficacious method for treating, inhibiting or
preventing arterial restenosis resulting from excessive
intimal hyperplasia. Excessive intimal hyperplasia and
30 arterial restenosis frequently occur following vascular
interventional procedures such as angioplasty of any
vessel (e.g., carotid, femoral, coronary, etc.); or any
coronary revascularization procedure, including balloon

angioplasty, laser angioplasty, c r nary artery bypass
grafting, atherectomy r cor nary artery st nts.

     Inhaled NO may be used, according to this
invention, to treat a mammal that has been identified as
5 having an existing arterial restenosis. Mammals are
known to display various signs and symptoms relating to
the existence of an arterial restenosis, and may be
identified thereby. The recognition of such signs and
symptoms is within the skill of medical practitioners.
10 Signs and symptoms of a arterial restenosis in a mammal
include, but are not limited to, the following: chest
pain, shortness of breath, electrocardiographic changes
and coronary angiographic findings. The objective of
treating an existing arterial restenosis with inhaled NO
15 is to reduce the thickness of the intima of a restenosed
artery, so as to increase the diameter of the arterial
lumen, i.e., reduce the pathological stricture.

     Alternatively, inhaled NO may be used, according
to this invention, to inhibit or prevent arterial
20 restenosis in a mammal (e.g., a human) that does not have
an existing arterial restenosis but has been identified
as being at risk of developing an arterial restenosis.
Certain vascular interventional procedures, e.g., PTCA
and coronary bypass surgery, can cause arterial trauma.
25 Arterial trauma is known to lead to excessive intimal
hyperplasia and arterial restenosis. Therefore, those
procedures put a patient at risk of developing an
arterial restenosis. Accordingly, individuals who have
undergone one or more vascular interventional procedures,
30 and individuals for whom one or more vascular
interventional procedures are imminent, may be
advantageously treated according to this invention.

     The inhaled NO is, for example, administered to a
human patient who is scheduled to undergo a vascular
35 interventional procedure. Inhaled NO may be administered

in advance f a vascular interventional procedure, to
minimize the amount of intimal thickening that takes
place following the arterial trauma normally associated
with vascular interventional procedures. A patient
5 receiving inhaled NO as a preventive measure before a
vascular interventional procedure may or may not have an
existing arterial restenosis. Alternatively, inhaled NO
may be administered to a patient who has already
undergone a vascular interventional procedure. In that
10 situation, administration of the inhaled NO preferably
will begin within hours of the vascular interventional
procedure. Of course, one of skill in the art will
recognize that inhaled NO may be administered before,
during and after a vascular interventional procedure.
15 Regardless of whether the inhaled NO is administered
before, during or after the procedure, or all three, the
inhaled NO may be administered continuously or
intermittently.

Administration of Inhaled NO

20   Inhaled NO is preferably administered from a
source of stored, compressed NO gas. Compressed NO gas
may be obtained from a commercial supplier such as Airco
(Murray Hill, NJ), typically as a mixture of 200-800 ppm
NO in pure N_2 gas. The source of NO can be 100% NO, or
25 diluted with N_2 or any other inert gas (e.g., helium).
It is vital that the NO be obtained and stored as a
mixture free of any contaminating O_2 or higher oxides of
nitrogen, because such higher oxides of nitrogen (which
can form by reaction of O_2 with NO) are potentially
30 harmful to lung tissues. If desired, purity of the NO
may be demonstrated with chemiluminescence analysis,
using known methods, prior to administration to the
patient. Chemiluminescence NO-NO_x analyzers are
commercially available (e.g., Model 14A, Thermo

Environmental Instruments, Franklin, MA). The NO-N$_2$
mixture may be blended with air r O$_2$ through, for
example, calibrated rotameters which have been validated
previously with a spirometer. The final concentration of
5 NO in the breathing mixture may be verified with a
chemical or chemiluminescence technique well known to
those in the field (e.g., Fontijin et al., Anal. Chem.
42:575 (1970)). Alternatively, NO and NO$_2$ concentrations
may be monitored by means of an electrochemical analyzer.
10 Any impurities such as NO$_2$ can be scrubbed by exposure to
NaOH solutions, baralyme, or sodalime. As an additional
control, the FiO$_2$ of the final gas mixture may also be
assessed. If desired, the ventilator may have a gas
scavenger added to the expiratory outlet to ensure that
15 significant amounts of NO will not escape into the
adjacent environment.

In a hospital or emergency field situation,
administration of NO gas could be accomplished, for
example, by attaching a tank of compressed NO gas in N$_2$,
20 and a second tank of oxygen or an oxygen/N$_2$ mixture, to
an inhaler designed to mix gas from two sources; by
controlling the flow of gas from each source, the
concentration of NO inhaled by the patient can be
maintained at an optimal level. NO gas may also be mixed
25 with room air, using a standard low-flow blender (e.g.,
Bird Blender, Palm Springs, CA). NO may be generated
from N$_2$ and O$_2$ (i.e., air) by using an electric NO
generator. Such a generator is described in Zapol U.S.
patent application 07/850,383 (notice of allowance
30 issued), which is hereby incorporated by reference. In
addition, NO may be provided intermittently from an
inhaler. The use of an inhaler may be particularly
advantageous if a phosphodiesterase inhibitor is
administered, orally or by inhalation, in conjunction
35 with the NO.

NO may be administered to a mammal identified as
having a thrombosis or arterial resten sis,   r a mammal
identified as being at risk for developing a thrombosis
or arterial restenosis, at a concentration of from 0.1
5 ppm to 300 ppm in air, pure oxygen, or another suitable
gas or gas mixture, for as long as needed. Preferably,
the concentration will be between 1.0 and 200 ppm; and
most preferably between 20 and 100 ppm (e.g., 30 ppm, 40
ppm,
10 50 ppm, 60 ppm or 80 ppm). The concentration may
temporarily increased for short periods of time: e.g.,
5 min at 300 ppm NO, when an immediate dramatic effect is
desired. For the reasons explained below, concomitant
treatment with a phosphodiesterase inhibitor may decrease
15 the total dosage of NO required (or allow intermittent
dosage) to produce a satisfactory anti-thrombotic or
anti-restenosis effect.

        For treatment, inhibition or prevention of
arterial restenosis, it may be necessary to administer
20 inhaled NO by nasal prongs, mask, tent, intra-tracheal
catheter or endotracheal tube, for an extended period,
i.e., days or weeks. The administration may be
continuous, during the extended period. Alternatively,
administration could be intermittent during the extended
25 period. The administration of gaseous NO may be via
spontaneous or mechanical ventilation.

Assessment of Effects of Inhaled NO
        When inhaled NO is administered to treat, inhibit
or prevent thrombosis or arterial restenosis, it is
30 desirable to monitor the effects of the NO inhalation.
Such monitoring can be used, in a particular individual,
to verify desirable effects and to identify undesirable
side effects that might occur. Such monitoring is also

- 14 -

useful in adjusting dose level, durati n and frequency of
administration f inhaled NO in a given individual.
        Preferably, the effects of inhaled NO on a patient
would be assessed by one or more of the following:
5 clinical manifestations such as chest pain;
   electrocardiography; serial analyses of vascular patency
   by ultrasound, coronary angiography or other means; and
   increases in levels of cGMP in plasma or platelets.

Phosphodiesterase Inhibitors

10   NO decomposes rapidly by reacting with molecular
   oxygen to produce nitrite and nitrate. In addition, NO
   entering the blood is rapidly inactivated by tight
   binding to hemoglobin. For these reasons, NO has only a
   short half-life in arterial blood. This means that
15 inhaled NO advantageously avoids systemic vasodilation,
   an undesirable, potentially dangerous side effect
   associated with sustained systemic NO release from NO
   donor compounds.
        It may be desirable to prolong the beneficial
20 effects of inhaled NO within the target cells or within
   cells interacting with the target cells in the lung. In
   the context of thrombosis inhibition, prevention and
   treatment, circulating platelets are the target cells.
   In the context of arterial restenosis inhibition,
25 prevention and treatment, circulating platelets (and
   possibly white cells) are the cells which interact with
   the target cells in the vasculature.
        In determining how to prolong the beneficial
   effects of inhaled NO, it is useful to consider that one
30 of NO's _in vivo_ effects is activation of soluble
   guanylate cyclase, which stimulates production of
   guanosine-3',5'-cyclic monophosphate (cGMP). At least
   some of the beneficial effects of NO appear to result
   from NO's stimulation of cGMP biosynthesis. Accordingly,

- 15 -

in a preferred embodiment f the inventi n, a
ph sphodi sterase inhibit r is administered in
conjunction with NO inhalation, to inhibit the breakdown
of cGMP by endogenous phosphodiesterases.

5   The phosphodiesterase inhibitor may be introduced
into the mammal by any suitable method, including via an
oral, transmucosal, intravenous, intramuscular,
subcutaneous or intraperitoneal route. Alternatively,
the inhibitor may be inhaled by the mammal. For

10 inhalation, the phosphodiesterase inhibitor is
advantageously formulated as a dry powder or an
aerosolized solution having a particle or droplet size of
less than 10 µm for optimal deposition in the alveoli,
and may optionally be inhaled in a gas containing NO.

15   A preferred phosphodiesterase inhibitor is
Zaprinast" (M&B 22948; 2-o-propoxyphenyl-8-azapurine-6-
one; Rhone-Poulenc Rorer, Dagenham Essex, UK).
Zaprinast" selectively inhibits the hydrolysis of cGMP
with minimal effects on the breakdown of cAMP in vascular

20 smooth muscle cells (Trapani et al., J. Pharmacol. Exp.
Ther. 258:269 (1991); Harris et al., J. Pharmacol. Exp.
Ther. 249:394 (1989); Lugnier et al., Biochem. Pharmacol.
35:1743 (1986); Souness et al., Br. J. Pharmacol. 98:725
(1989)). When using Zaprinast" according to this

25 invention, the preferred routes of administration are
intravenous or oral. The suitable dose range may be
determined by one of ordinary skill in the art. A stock
solution of Zaprinast" may be prepared in 0.05 N NaOH.
The stock can then be diluted with Ringer's lactate

30 solution to the desired final Zaprinast" concentration,
immediately before use.

          This invention may be practiced with other
phosphodiesterase inhibitors. Various phosphodiesterase
inhibitors are known in the art, including dipyridamole

35 and theophyline. As with Zaprinast", the route of

administration and suitable dose range may be determined
by one of ordinary skill in the art.

Other Antithrombotic Agents in Conjunction with Inhaled NO

5   Thrombosis may be treated by agents that inhibit
thrombus formation, agents that stimulate thrombolysis,
i.e., thrombus dissolution, or both. Examples of
antithrombotic agents are aspirin, streptokinase,
urokinase, tissue plasminogen activator ("t-PA"), met-t-
10 PA (i.e., t-PA with an N-terminal methionine residue),
FE1X (a t-PA analog) heparin, hirudin and Hirulog" (a
hirudin analog). Other antithrombotic agents could also
be used in the practice of this invention. One or more
such antithrombotic agents may be administered to a
15 mammal before, during or after treatment with inhaled NO,
so that their separate antithrombotic activity is
advantageously used to augment the antithrombotic
effect(s) of inhaled NO.
              For example, in one embodiment of this invention,
20 an appropriate dose of t-PA is administered before,
during or immediately after NO inhalation, to treat
thrombosis. While the inhaled NO is inhibiting or
preventing the formation of new thrombi, the t-PA will
stimulate the dissolution of: (1) thrombi already
25 present at the time inhaled NO was administered, or (2)
thrombi formed (albeit at a reduced rate) during or
immediately after the NO inhalation. The selection of
appropriate antithrombotic agents to be administered in
conjunction with inhaled NO, and the selection of the
30 appropriate dosage and route of administration of those
antithrombotic agents is within ordinary skill in the
art.
              In a preferred embodiment of this invention,
aspirin is administered before, during or immediately

after NO inhalation, to treat thrombosis. The preferred
aspirin dose range is 81 to 325 mg (rally) per day.

   While the two aspects of this invention (i.e.,
thrombosis and arterial restenosis) have been described
5 separately, it should be appreciated that an individual
may already have, or be at risk of developing, both a
thrombosis and arterial restenosis. Under such
circumstances, both aspects of the invention could be
practiced at the same time, in the same individual, with
10 a single administration of inhaled NO.

Experimental Information
Thrombosis Experiments

   The antiplatelet effects of NO have been assessed
in a recognized canine model of acute coronary
15 thrombolysis after thrombus formation. The canine model
used was essentially as described by Yasuda et al. (J.
Am. Coll. Cardiol. 13:1409 (1989)) and Gold et al.
(Circulation (suppl. IV) 83:IV26 (1991)).

   Twenty-five adult mongrel dogs (20-25 kg) of
20 either sex were anesthetized with pentobarbital (30 mg/kg
body weight). Supplemental pentobarbital was
administered as required to maintain general anesthesia.
The dogs' tracheas were intubated and their lungs were
mechanically ventilated at 15 breath/min and 10-15 ml/kg
25 with a ventilator at FiO_2 within 21-35% (Hudson
Ventronics, Tenecula, CA) and adjusted to maintain the
arterial blood O_2 between 80 and 100 torr. The oxygen
saturation was continuously monitored during the
experiment, using a pulse oximeter (Nellcor, Inc.,
30 Haywood, CA).

   The femoral artery and vein were cannulated with a
polyvinyl chloride catheter for continuous arterial
pressure, blood sampling and infusion. Lidocaine 0.1
mg/kg/min i.v., was given when arrhythmias occurred.

- 18 -

Th racotomy was performed thr ugh the left fifth
intercostal space. Th pericardium was opened and
suspended to create a pericardial cradle. The left
anterior descending artery was dissected out free, and a
5 2.5 cm segment was isolated distal to the first diagonal
branch. One milliliter of blood was withdrawn for
thrombus formation. A 0.7 mm i.d. catheter was inserted
into a side branch of the isolated left anterior
descending coronary artery segment, and an ultrasonic
10 flow probe (T101 Transonic System, Inc., Ithaca, NY) was
placed on the proximal portion of the artery for
continuous blood flow monitoring. A 2 mm wide plastic
wire (Mass Gas and Electric Supply, Watertown, MA) was
progressively constricted around the left anterior
15 descending artery, just distal to the proposed site of
thrombus formation, to limit blood flow to 50 ± 10% of
baseline. Previous angiographic study has shown this to
decrease the luminal diameter by more than 90%.
        The isolated left anterior descending coronary
20 artery was traumatized by four consecutive external
compressions with blunted forceps during 3-5 seconds, to
damage the endothelium and promote thrombus adherence.
Snare occlusions were made distal to the probe and
proximal to the constriction site. Thrombin (0.1 ml of
25 100 units/ml; Thrombinar, Armour Pharmaceutical,
Kankakee, IL) mixed with 0.3 ml of blood was injected
through the side branch catheter into the emptied
coronary artery segment to induce thrombus formation.
After 10 minutes, the proximal snare was released. Two
30 minutes later, the distal snare was released. Ten
minutes after thrombus formation, a heparin bolus (75
UI/kg) was administered intravenously and followed by a
continuous heparin infusion (50 UI/kg/h). After a 30-
minute period of stable occlusion, recombinant

- 19 -

t-PA boluses (0.45 mg/kg; Activase™, Genentech Inc.,
South San Francisco, CA) were administered at 15-minut
intervals, until recanalization of the thrombosed
coronary artery was achieved or a maximum of four boluses
5   had been administered.

This procedure induced alternating periods of
recanalization and reocclusion (defined as less than 25%
of poststenotic flow) after initial reflow of the left
descending coronary artery. The ratio opening time/total
10 time was recorded as the primary outcome variable. ECG,
systemic arterial pressure, left atrial pressure and
coronary blood flow were continuously recorded. Animals
with: (1) no reperfusion, (2) reperfusion without
occlusion, (3) fewer than 3 cycles during the first 45-
15 minute observation period, or (4) death before the end of
the first observation period, were excluded from further
study.

NO gas (800 ppm NO in nitrogen, Airco, Murray
Hill, NJ) was mixed with room air using a standard low-
20 flow blender (Bird Blender, Palm Springs, CA) and then
titrated with varying quantities of NO, to maintain a
constant FiO_2 just prior to delivery to the ventilator.
Inspired NO level was continuously monitored by a
chemiluminescence NO-NO_x analyzer (Model 14A, Thermo
25 Environmental Instruments, Franklin, MA; Fontijin et al.,
supra). The FiO_2 was measured (oxygen meter No. 5590,
Hudson, Temecula, Calif.) distal to the reservoir bag
after the NO-containing gases were mixed. The exhaled
gases, as well as those discharged from the
30 chemiluminescence analyzer, were scavenged by use of a
venturi exhalation trap maintained at negative
atmospheric pressured by the laboratory's central vacuum
system. The ambient NO/NO_2 levels, as measured
intermittently by chemiluminescence, did not increase
35 during the experiments. At each time samples were taken

- 20 -

and methemoglobin levels were measured. Heart rate,
bl od pr ssure and cor nary flow were monitored and
recorded continuously.

After a 45 minute baseline study period
5 (pretreatment period) the animals were divided into four
groups: Group A (n=6) was given 0 ppm inhaled NO (i.e.,
air) for 45 minutes; Group B (n=6) was given 20 ppm
inhaled NO for 45 minutes; Group C (n=6) was given 80 ppm
inhaled NO for 45 minutes and Group D (n=6) was given 200
10 ppm for 45 minutes. All groups of animals were then
observed for a third study period beginning with the
cessation of NO administration and lasting 45 minutes
(posttreatment period).

In Vitro Measurement of Platelet Aggregation

15   Blood from 8 dogs was collected in 0.01M citrate
and centrifuged at room temperature at 370 x g for 5
minutes, for the preparation of platelet-rich plasma
(PRP), and at 1200 x g for 10 minutes, for the
preparation of platelet-poor plasma (PPP). PRP was
20 exposed only to plastic containers or, during testing,
siliconized glassware. The platelet count
(Thrombocounter C* platelet counter, Coulter Electronics,
Inc., Hialeah, FL) of PRP was adjusted by dilution with
PPP to obtain 300,000/mm^3±10%. PRP was then aliquoted
25 into cuvettes incubated at 37°C with magnetic stirring
(1000 rpm) in a dual channel aggregometer (Model 440,
Chrono-Log Corp., Havertown, PA). Light transmission was
continuously recorded on a recorder (Model 707, Chrono-
Log Corp., Havertown, PA). The aggregation sample tube
30 was obstructed with a rubber cap and two 19 gauge needles
(Sherwood Medical, St. Louis, MO) were placed through
this cap, allowing the delivery of a mixture of NO gas
and oxygen above the PRP. The inlet needle was connected
via a flowmeter to a gas reservoir into which a mixture

- 21 -

of NO gas, air and oxygen titrated as d scribed above was
delivered. The NO c nc ntrati n was c ntinu usly
monitored by the chemiluminescence NO-NO$_x$ analyzer. A
needle valve allowed regulation of the flow through the
5   sample to approximately 40 ml/min. Positive pressure was
maintained in the system by having the outlet needle
connected to a recipient flask filled with 5 cm of water
in which constant bubbling was maintained.

        ADP-induced platelet aggregometry studies were
10 performed after the exposure of the test cuvette
containing 450μl of adjusted PRP to the NO gas mixture
for 10 minutes. Gas administration was continued during
the measurement of ADP-induced aggregation. Platelet
aggregation was studied using different aliquots of PRP
15 treated with 20, 80, 200 and 400 ppm of NO, in random
order. Control ADP-induced platelet aggregation studies
without NO were performed before and after the
administration of NO to assess the stability of the PRP
preparation. All experiments were completed within 4
20 hours of blood collection.

Data Analysis

        Artery patency was defined as the fraction of the
total observation period during which flow was greater
than 25% of the basic flow after the initial stenosis was
25 created. Except as noted, results are expressed as mean
± SEM. The significance of differences between groups
was determined with Student's t-test for paired or
unpaired values as appropriate. The significance of the
dose response of the effect of NO on ADP-induced platelet
30 aggregation was assessed with two-way analysis of
variance. A p value <0.05 was considered significant.

Canine Thrombolysis and Reperfusion Model Results

In each experimental group, the external
constrictor reduced left anterior descending artery blood
flow by 54 ± 2% of baseline, from 22 ± 2 to 10 ± 1
5   ml/min.   The median number of tPA boluses required to
obtain reperfusion was two, with a range of 1 to 4 (Table
1). Cyclic reflow and reocclusion accompanied by
electrocardiographic evidence of myocardial injury
occurred in all animals except one in which reocclusion
10 did not occur; this animal was excluded from further
study.

TABLE 1

Baseline Values of Physiological Parameters

Parameter Measured	Group A 0 ppm NO	Group B 20 ppm NO	Group C 80 ppm NO	Group D 200 ppm NO
	(n=6)	(n=6)	(n=6)	(n=6)
Post-stenotic flow (% before stenosis)	51.7±3.5	55.2±3.7	51.6±4.7	54.6±1.9
Number of tPA boluses	2.0±0.4	1.7±0.2	1.8±0.3	2.0±0.4
ACT (sec)	219±16	219±14	236±5.3	196±12.8
FIO$_2$(%)	29.0±1.1	27.5±1.0	32.2±1.5	31.6±3.0
Ratio (%)	55.7±8.5	50.6±6.9	48.8±2.9	45.2±5.6
Hb(mg/dl)	13.26±1.35	14.28±0.85	14.08±0.85	12.95±0.64
Platelet ct (x10^3/mm^3)	301±55	292±25	277±35	237±35
MetHb (%)	0.25±0.17	0.10±0.06	0.18±0.09	0.26±0.09

- 24 -

In Tabl 1: "Number of tPA boluses" ref rs to number of boluses injected; "ACT" refers to activated clotting time; "FIO$_2$" refers to inspired O$_2$ fraction; "Ratio" refers to unoccluded/occluded coronary artery at
5 baseline; "Platelet Ct" refers to platelet count; and "MetHb" refers to methemoglobin (percentage of total hemoglobin). There is no significant difference between the baseline flow rate ratios at 0 ppm (control), 20 ppm, 80 ppm and 200 ppm of inhaled NO (n=6 for each group).

10 Effects of Inhaled NO on Artery Patency
In animals receiving 0 ppm inhaled NO (Group A), arterial patency did not change during the three treatment periods (Fig. 1A). In animals receiving 20 ppm inhaled NO (Group B), arterial patency increased from
15 50.6 ± 6.9% during the pre-inhalation baseline period, to 63.8 ± 7.9% during the inhalation period (p < 0.01) (Fig. 1B). In animals receiving 80 ppm inhaled NO (Group C), arterial patency increased from 48.8 ± 2.9% during the pre-inhalation baseline period, to 75.1 ± 6.7% during the
20 inhalation period (p < 0.01) (Fig. 1C). In animals receiving 200 ppm inhaled NO (Group D), arterial patency increased from 45.27 ± 5.6% during the pre-inhalation baseline period to 54.8 ± 10.4% during the inhalation period (p = NS) (Fig. 1D). In group C the increased
25 arterial patency observed during the NO inhalation period persisted during the 45-minute post-inhalation period (70.1 ± 7.1% vs. baseline, p < 0.05).
When the inhalation period arterial patency results from groups B, C, and D were pooled, there was a
30 statistically significant difference between the pooled treatment groups and the control group (p < 0.005). When the 45-minute post-inhalation period artery patency results from groups B, C, and D were pooled, there was

also a statistically significant difference between the
p led treatment groups and the control gr up (p <
0.005).

5   Neither platelet count nor blood hemoglobin
changed during or after NO administration in any of the
groups of animals (Table 3). Methemoglobin levels
increased from $0.2 \pm 0.1\%$ to $1.1 \pm 0.4\%$ in the dogs
breathing 200 ppm inhaled NO (Table 3). This increase
persisted after the NO administration was stopped and was
10 not seen in dogs receiving lower doses of NO.

       Systemic arterial pressure decreased in all groups
of dogs during this study, and did not differ between the
dogs receiving NO and those who did not (Table 2A). Left
atrial pressure was unchanged in all groups of animals
15 throughout the duration of the study (Table 2B).

TABLE 2A

Mean Systemic Arterial Pressure (SAP) (mmHG)
Before, During and After NO Inhalation

NO Treatment	Before	During	After
0 ppm NO (N=6) Group A	89±8	82±6	80±7
20 ppm NO (n=6) Group B	105±6	96±6	82±6
80 ppm NO (n=6) Group C	91±5	81±9	78±7
200 ppm NO (n=6) Group D	94±3	95±4	93±4

values are expressed as mean±SEM.

TABLE 2B

Mean Left Atrium Pressure (LAP) (mmHG)
Before, During and After NO Inhalation

NO Treatment	Before	During	After
0 ppm NO (N=6) Group A	5.5±0.6	5.4±0.7	5.4±0.7
20 ppm NO (n=6) Group B	5.2±1.6	4.7±1.4	4.6±1.1
80 ppm NO (n=6) Group C	5.3±0.6	5.2±0.6	4.9±0.6
200 ppm NO (n=6) Group D	4.6±1.1	4.2±0.9	3.7±0.8

Values are expressed as mean±SEM.

Effects of NO on ADP-Induced In Vitro Platelet
Aggregation

Addition of 20, 80, 200 and 400 ppm NO to the gas
mixture above the PRP led to a dose-related decrease in
the maximal change in light transmission caused by ADP
5 (Fig. 3). There was no change in the ADP-induced
decrease in light transmission in the two control
aggregation curves performed at the beginning and end of
each study (30.57±5.57% vs. 31.1±4.31%).

TABLE 3

Hematologic Values Before, During and After NO Inhalation

NO Treatment	Measurement	Before	During	After
0 ppm NO Group A (n=6)	Hb (mg/dl)	13.2±1.3	13.7±0.8	12.2±0.7
	Platelet Count (x10³/mm³)	301±55	284±53	276±35
	MetHb (%)	0.22±0.2	0.2±0.1	0.2±0.2
20 ppm NO Group B (n=6)	Hb (mg/dl)	14.3±0.85	12.7±0.7	2±1
	Platelet Count (x10³/mm³)	292±25	269±22	253±16
	MetHb (%)	0.1±0.1	0.4±0.2	0.1±0.02
80 ppm NO Group C (n=6)	Hb (mg/dl)	14.0±0.8	11.9±0.3	11.1±0.6
	Platelet Count (x10³/mm³)	277±35	238±32	209±37
	MetHb (%)	0.2±0.1	0.4±0.1	0.3±0.1

        The above results dem nstrat that inhal d NO
increases coronary artery patency after lysis of a
thrombus at the site of a critical stenosis without
producing any systemic hemodynamic effects. They also
5 show that the antithrombotic effects of inhaled NO
persist for at least 45 minutes after cessation of NO
inhalation. At NO concentrations similar to those
obtained in vivo, gaseous NO markedly inhibited ADP-
induced platelet aggregation in vitro.

10 Intimal Hyperplasia Experiments
        Neointimal smooth muscle cell hyperplasia is the
main pathologic process in human arterial restenosis.
Therefore, to elucidate the effect of inhaled NO on the
process of restenosis after a vascular interventional
15 procedure such as PTCA, the effect of inhaled NO on
intimal hyperplasia was studied, using a rat carotid
artery model of arterial injury (Clowes et al.,
Laboratory Invest. 49:327 (1983)), a standard animal
model for human neointimal smooth muscle cell
20 hyperplasia.

   Arterial Injury
        Adult male Sprague-Dawley rats (Charles River
Laboratories, Wilmington, MA) underwent balloon injury of
the common carotid artery in a manner previously shown to
25 bring about neointimal hyperplasia (Clowes et al.,
supra). Rats weighing 300 to 350 g were anesthetized by
intraperitoneal injection of ketamine (60-80 mg/kg) and
acepromazine (0.1 mg/kg). Once satisfactory anesthesia
had been achieved, a longitudinal midline incision was
30 made and the left carotid artery was isolated via blunt
dissection. After further careful dissection of the
internal and external carotid bifurcation, the distal
external carotid segment was ligated with a 4-0 silk

sutur . A small arteriot my was made in the external
carotid using microdissecting scissors and a 2 French
Fogarty balloon catheter (Baxter Edwards LIS) was
inserted through the arteriotomy and advanced
5   approximately two centimeters below the carotid
bifurcation. The balloon was filled with enough saline
to cause visible distention of the common carotid artery
and gently withdrawn to the level of the bifurcation.
The balloon catheter was then withdrawn and after
10 allowing back bleeding through the arteriotomy site to
eliminate potential thrombus and air bubbles, the
external carotid was ligated proximal to the arteriotomy
site using 4-0 silk sutures. After visual inspection to
insure adequate pulsation of the common carotid artery,
15 the surgical lesion was closed and the animals were
allowed to recover from anesthesia.

Chronic NO Inhalation

        Chronic NO inhalation was carried out in specially
prepared 40 liter acrylic inhalation chambers. The gas
20 mixtures were blended using separately regulated and
calibrated flow meters for oxygen, pressurized air and NO
stock gases (800 ppm and 10,000 ppm NO in N_2, Airco,
Murray Hill, NJ). The effluent gas from the chamber was
analyzed periodically throughout the experimental period,
25 to ensure stable FIO_2 and NO levels. The FIO_2 was
measured using a polarographic electrode (Hudson Oxygen
Meter 5590, Temecula, CA); NO concentration was measured
by chemiluminescence (Model 14A, Thermo Environmental
Instruments, Inc., Franklin, MA); and nitrogen dioxide
30 and nitrogen with higher oxidation states (NO_x) were
measured by chemiluminescence following conversion of the
NO_x to NO by a heated stainless steel oven (850°C) with
98% efficiency (Model 100B NO_x generator, Thermo
Environmental Instruments, Inc., Franklin, MA). Fresh

- 32 -

soda lime was maintained in the chamb rs to reduce NO_x.
NO c ncentrati n was maintained at 20 to 80 ppm,
depending upon the experiment. Serial measurements
revealed FIO_2 to be 21% and NO_x to be 3-4 ppm. The gases
5 exiting the exposure chambers, as well as those
discharging from the chemiluminescence instrument, were
scavenged using a venturi trap maintained at negative
atmospheric pressure with reference to the laboratory's
central vacuum system.

10   Control animals were maintained in filtered cages
in the same room as the NO-treated animals. The NO
exposure was begun 30 to 120 minutes prior to the
surgical procedure. During the surgical procedure, the
NO-treated group was taken out of the chamber and exposed
15 to NO by a modified face mask fed from the inflow gas
tubing of the chambers. Inhaled gas analysis revealed NO
levels of 50-80 ppm during the 80 ppm experiments and 10-
20 ppm during the 20 ppm surgical experiments. The total
time each animal was out of the chamber and under the
20 face mask ranged from 20 to 40 minutes.

Morphometric Analysis

         At 1, 3 and 14 days after balloon injury, the rats
were euthanized by lethal intraperitoneal injection of
sodium pentobarbital or ketamine. A 16 gauge catheter
25 was introduced into the ascending aorta via the left
ventricular apex. The descending thoracic aorta was
ligated. The animal was then perfused at a pressure of
100 mm Hg with 100 ml of normal saline followed by 50 ml
of 2% paraformaldehyde in phosphate buffered saline
30 (PBS). After in vivo fixation for 15 to 30 minutes, both
common carotid arteries were isolated and underwent
further overnight fixation by immersion in 1%
paraformaldehyde in PBS. The tissue was dehydrated using
sequentially increasing concentrations of ethanol

followed by xylene, and th n embedded in paraffin. Cr ss
secti ns (6 μm) were cut and stained with hemat xylin and
eosin and/or elastin for analysis. A single section 7 to
8 mm proximal to the carotid bifurcation was used for
5 analysis in each animal. The section being analyzed was
photographed at 100X. The image was digitized using a
Kodak 2135ᵘ scanner. The intimal and medial area
analyses were performed on a Power Macintosh 8100/80ᵘ
computer using the public domain NIH Image program
10 (written by Wayne Rasband at the U.S. National Institutes
Of Health and available from the Internet by anonymous
ftp from zippy.nimh.nih.gov or on floppy disk from NTIS,
5285 Port Royal Rd., Springfield, VA 22161; part number
PB93-504868). The morphometric analysis was performed
15 with the investigator blinded as to the experimental
group.

Statistical Analysis

        Statistical analysis was carried out utilizing
commercially available Statviewᵘ (Abacus Concepts)
20 software for Macintoshᵘ computers. The experimental
group was compared with the control group using an
unpaired two tailed t-test. A value of $P < 0.05$ was
considered statistically significant. All data are
represented as mean +/- SEM. The n value refers to the
25 number of animals per group.

Results from Rodent Model of Restenosis

        A total of 62 rats underwent the carotid injury
procedure. Five of the rats died due to vascular
complications during the arteriotomy or the balloon
30 injury, giving a procedure mortality rate of 8%. There
was no statistically significant maldistribution of the
deaths among the different experimental groups. There
were no post-procedure deaths among the 57 rats who

survived the initial procedure. All rats, regardless f
their xperimental group, exhibited normal gr oming
behavior and activity levels throughout the study. Other
than occasional ptosis on the side of the carotid injury,
5 no rats exhibited a gross neurological deficit. Analysis
of a subset of the experimental groups revealed weight
gain without statistical difference during exposure to
air, NO at 20 ppm and NO at
80 ppm.

10   In the first series of experiments, the rats
undergoing carotid balloon injury were exposed to either
ambient air (n = 12) or 80 ppm NO (n = 13) in air,
throughout the duration of the study. The animals were
sacrificed 14 days after injury for morphometric analysis
15 of the injured carotid artery. Examination by light
microscopy revealed that three rats from the air
(control) group and one rat from the NO-treated group had
thrombotic occlusion of the lumen of the injured carotid.
These animals were excluded from further analysis. As
20 expected, blood vessels from both groups exhibited a loss
of endothelium and the development of neointimal
hyperplasia. Examination by light microscopy did not
reveal a qualitative difference in the cellular
morphology of the neointima (data not shown). Quantitive
25 analysis, however, revealed that the 80 ppm inhaled NO
treatment resulted in a 38% inhibition of neointimal
hyperplasia, i.e., intima/media ratio of 0.932 +/- 0.13
in 80 ppm animals, compared to 1.512 +/- 0.147 in
0 ppm NO (control) animals (P = 0.008) (Fig. 4).

30   In the next series of experiments, the effect of a
one week exposure to NO was tested. The experimental
group (n = 7) was exposed to 80 ppm NO for seven days and
then transferred to air for an additional seven days,
prior to sacrifice. The control group (n = 5) was
35 exposed to air for the entire 14 day period. There was

no qualitative morphologic difference between the groups.
In addition, quantitive analysis of the intima/m dia
ratio revealed no significant difference in neointimal
hyperplasia between the two groups, i.e., 1.059 +/- 0.239
5   in NO-treated animals, compared to 1.217 +/- 0.309 in
controls (P = not significant) (Fig. 4).

        In a third series of experiments, a lower inhaled
dose of NO was studied: the rats breathed either 20 ppm
NO (n = 8) or air (n = 8). Again, no quantitative or
10 qualitive difference in the degree of neointimal
hyperplasia could be found between the 2 groups, i.e.,
intima/media ratio of 0.917 +/- 0.233 for 20 ppm NO-
treated animals, compared to 0.985 +/- 0.149 for air-
breathing controls (P = not significant) (Fig. 4).
15   The above results demonstrate that inhalation of
80 ppm NO, over a two-week period, significantly inhibits
neointimal hyperplasia following balloon-induced injury
to carotid arteries, in a rodent model.
        Other embodiments of the invention are within the
20 following claims.

Claims

1. Use of gase us NO f r the manufacture of a
medicament for treatment or prev ntion of arterial
restenosis resulting from excessive intimal hyperplasia
5   in a mammal.

2. The use of claim 1, wherein the treatment or
prevention is by a method comprising the steps of:
    (a) identifying a mammal that has an existing
arterial restenosis or is at risk for developing arterial
10 restenosis; and
    (b) causing said mammal to inhale a
therapeutically effective concentration of gaseous NO.

3. The use of claim 1 or 2, wherein said mammal
is a human.

15   4. The use of claim 1 or 2, wherein said mammal
has undergone or is preparing to undergo a vascular
interventional procedure.

5. The use of claim 4, wherein said vascular
interventional procedure is selected from the group
20 consisting of angioplasty, coronary artery surgery and
coronary artery stents.

6. The use of claim 5, wherein said procedure is
percutaneous transluminal coronary angioplasty (PTCA).

7. The use of claim 1 or 2, wherein said NO is
25 inhaled in a predetermined concentration range.

8. The use of claim 7, wherein said concentration
range is 0.1 ppm to 300 ppm.

9.   The use of claim 1 or 2, wherein said gas us
NO is inhaled as a mixtur comprising NO, O_2, and N_2
gases.

10.   The use of claim 9, wherein said mixture
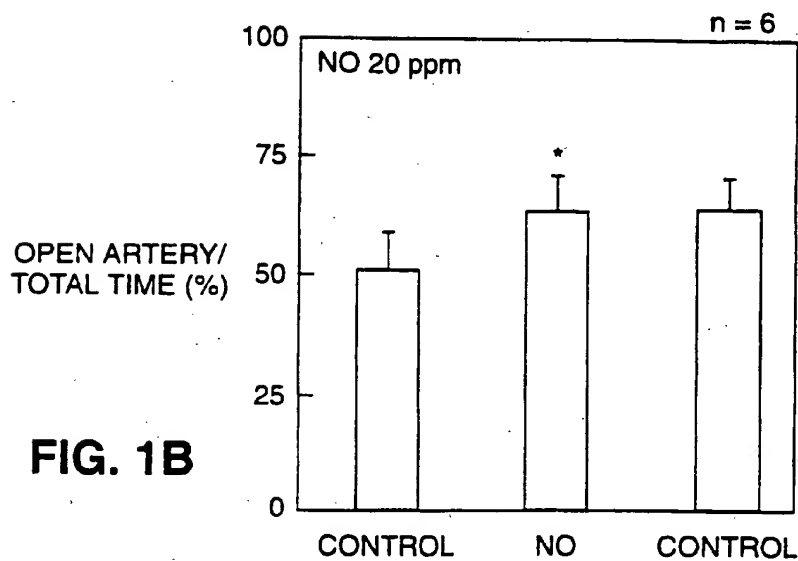5 comprises between 20% and 99% O_2 gas by volume.
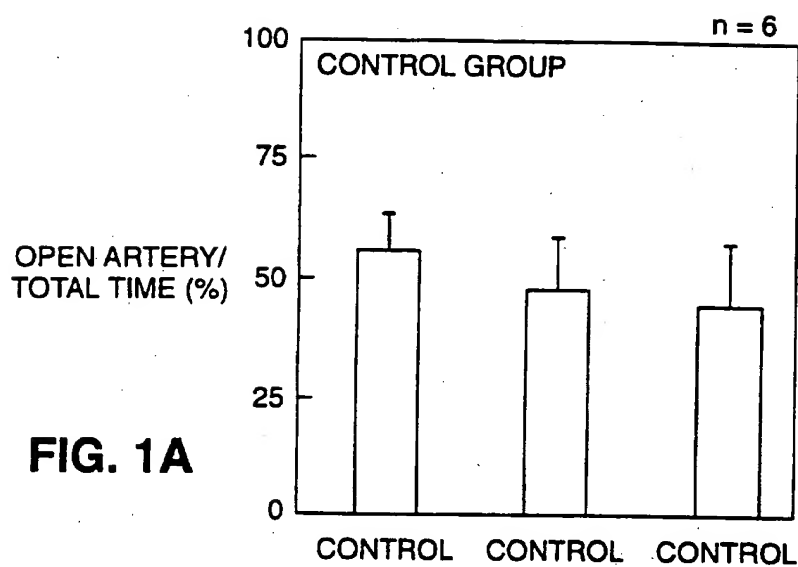
11.   The use of claim 9, wherein said O_2 is mixed
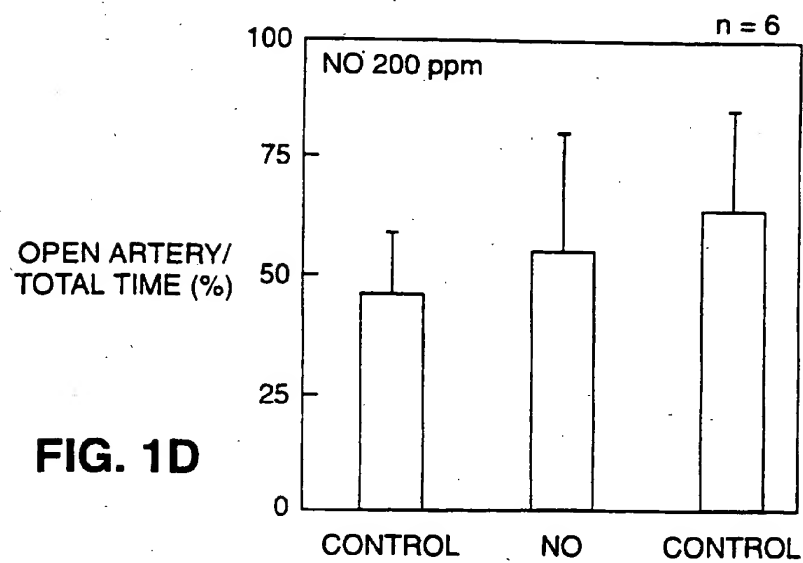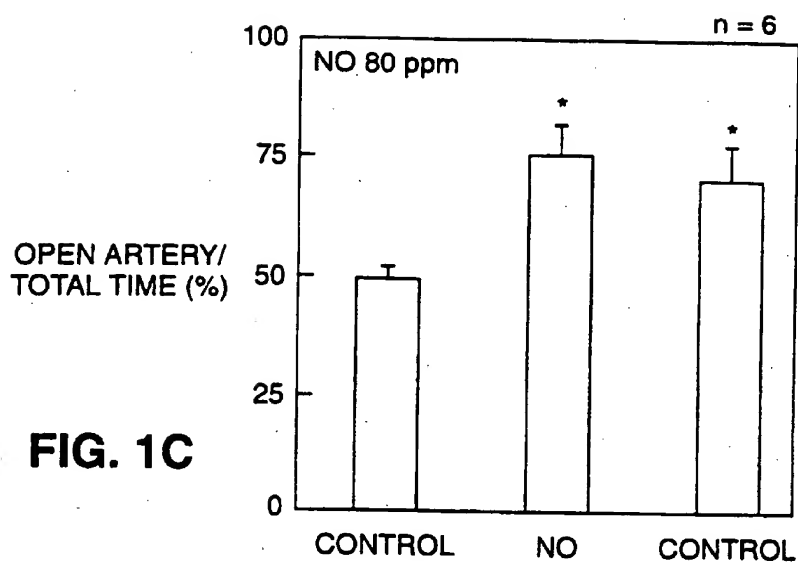with said NO immediately before said mixture is inhaled
by said mammal.

12.   The use of claim 1 or 2, further comprising
10 the step of administering to said mammal, in conjunction
with NO inhalation, a therapeutically effective amount of
a second compound that potentiates the therapeutic effect
of gaseous NO.

13.   The use of claim 12, wherein said second
15 compound is selected from the group consisting of a
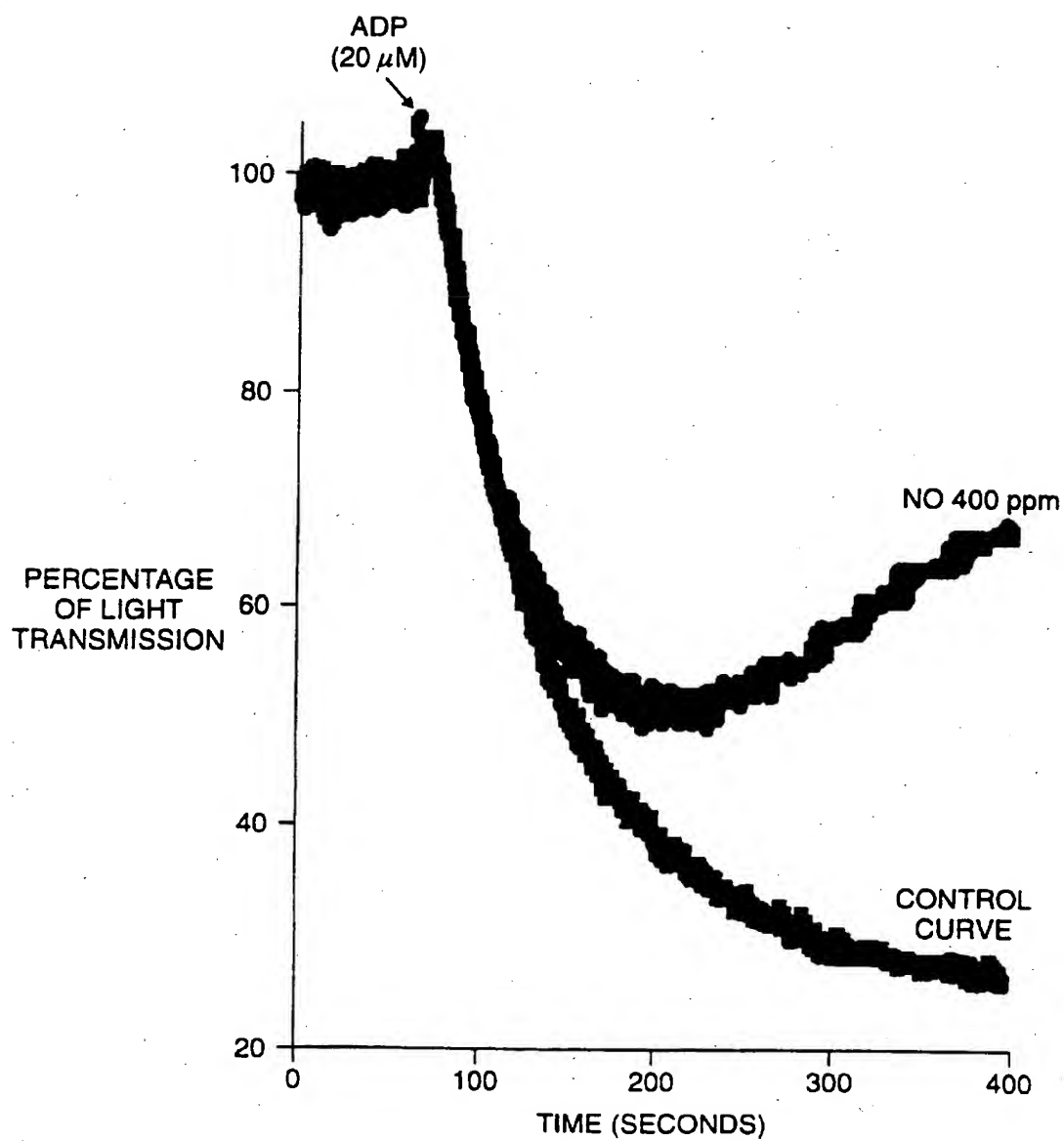phosphodiesterase inhibitor and superoxide dismutase.

14.   The use of claim 13, wherein said
phosphodiesterase inhibitor is selected from the group
consisting of 2-o-propoxyphenyl-8-azapurin-6-one
20 (Zaprinast"), dipyridamole and theophyline.

15.   The use of claim 1 or 2, wherein said gaseous
NO is inhaled in the absence of tobacco smoke.
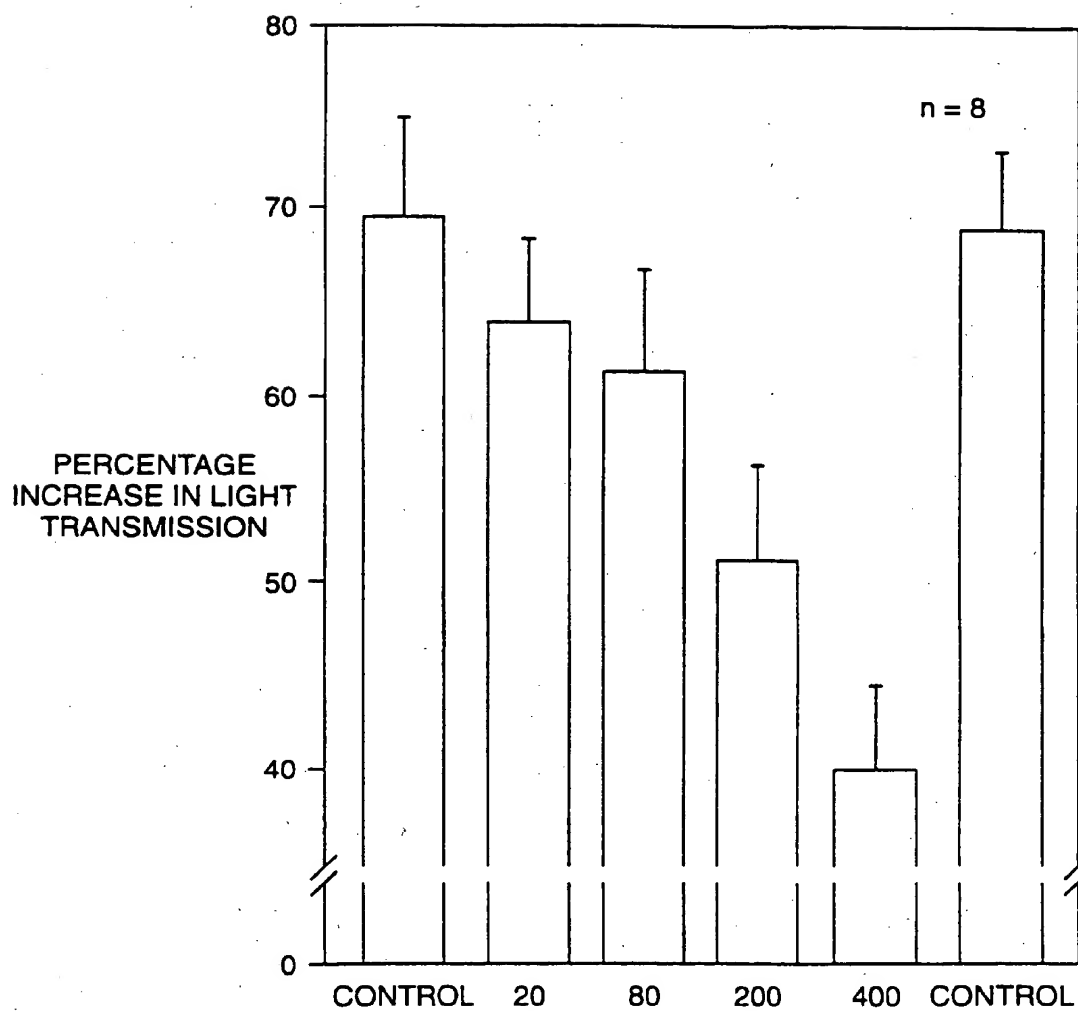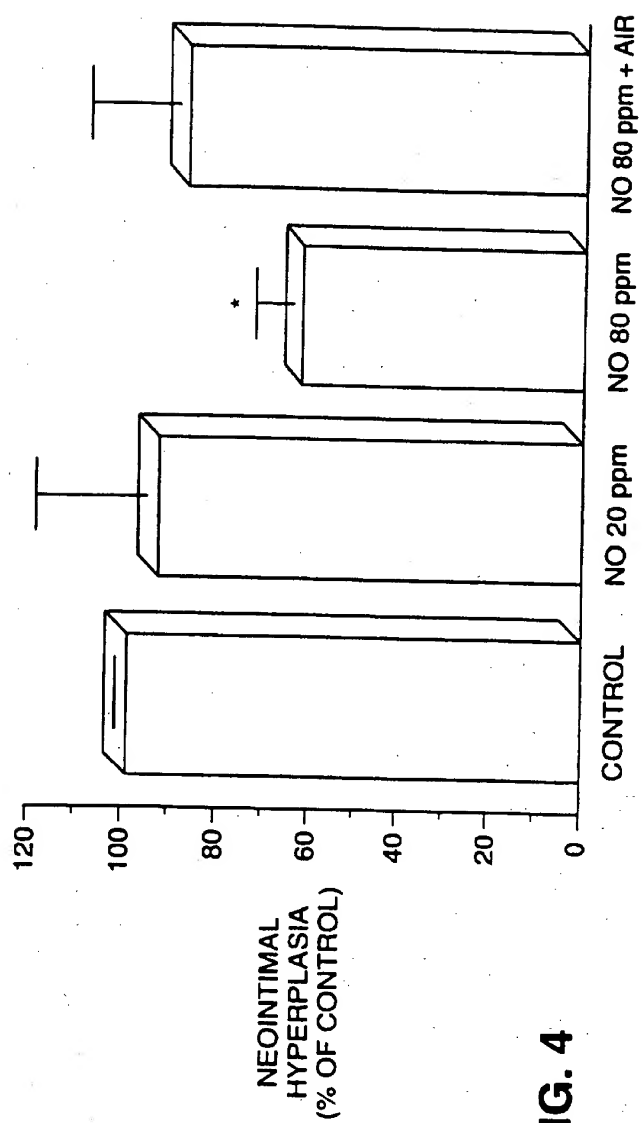
FIG. 1A

FIG. 1B

FIG. 1C

FIG. 1D

FIG. 2

FIG. 3

FIG. 4

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61L 9/04; A61K 9/12
US CL   :424/434, 44, 45; 514/957

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   424/434, 44, 45; 514/957

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US, A, 5,427,797 (FROSTELL ET AL) 27 June 1995, see the entire document.	1-15

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	
"E"	earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 MARCH 1996	**04 APR 1996**

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer FREDERICK KRASS
Facsimile No. (703) 305-3230	Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*